Ly-1 as a Differentiation Antigen on Normal and Neoplastic B Cells

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INTRODUCTION

A small subpopulation of splenic B cells carries the Ly-1 antigen, previously considered restricted to T cells (Hardy et al., 1982; Manohar et al.). The cells in this "Ly-1 B" subpopulation express other B lineage cell surface antigens, are detectable in spleen, are enriched in peritoneal washouts, are rare in (or absent from) lymph nodes and bone marrow and appear early during development (Hayakawa et al., 1983; Hayakawa et al., 1984). Thus Ly-1 B cells constitute a significant fraction of the Ig^+ cells in spleens of newborn (3-5 days) mice, but decrease to a minor population (1-2 percent of total lymphocytes) in adults.

Certain autoimmune mouse strains (NZB) have increased levels of Ly-1 B and this enlarged population is responsible for the large amounts of IgM secreted *in vitro* in the absence of exogenous antigen (Hayakawa et al., 1983). Moreover, such secreted IgM contains autoantibody, binding to thymocytes or ssDNA (Hayakawa et al., 1984). In normal strains such as BALB/c, the PFC generated in response to exogeneous antigens (such as SRBC, DNP-KLH and TNP-Ficoll) do not derive from Ly-1 B; however an autoantibody elicited by injection of LPS that lyses bromelain-treated mouse erythrocytes does come exclusively from Ly-1 B (Hayakawa et al., 1984).

Several years ago, Lanier noted the presence of Ly-1 on several B lymphomas and suggested that these might represent the transformation of a rare normal cell type. Recent studies with B cell tumor lines at various stages of differentiation (Davidson et al.) suggest that the Ly-1 marker is expresed on at least some B cells from pre-B to antibody forming cells. One of these lines, NFS-5, appears to be a $pre\text{-}B^+$ cell that expresses Ly-1 and spontaneously differentiates to a $Ly\text{-}1^+, IgM^+$ population *in vitro*. Another, NFS-1.0, appears similar by surface phenotype to the Ly-1 B population in NZB mice and this tumor can be triggered with LPS to make bromelain plaques. Curiously, many of these Ly-1 B cell lines express the lambda light chain, normally very rare in mice.

LY-1 EXPRESSION ON B CELLS IN NORMAL TISSUES

Quantitation of the number of Ly-1 bearing B cells in most normal tissues is complicated by the fact that the level of Ly-1 is quite low (compared with the level on T cells) and by the relative rarity of such cells. We have utilized three color immunofluorescence on a modified dual-laser Fluorescence Activated Cell Sorter (FACS) to quantitate the levels of three surface antigens on a cell-by-cell basis (Parks et al.; Hardy et al., 1983; Hardy et al, 1984). Thus we can stain cells simultaneously with antibodies specific for Ly-1, Ly-2 (an isotype-matched

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reagent control) and some B cell marker; cells that are Ly-1$^+$, Ly-2$^-$ should include all Ly-1 B cells in the cell suspension under study.

This analysis is carried out by staining bone marrow or spleen cell suspensions (from which erythrocytes have been lysed) with biotin-coupled (bi-) anti-Ly-1 (revealed by Texas Red-avidin), allophycocyanin-coupled (apc-) anti-Ly-2 and several different fluorescein-conjugated (Fl-) monoclonal antibodies specific for various B cell differentiation antigens. In addition, to avoid nonspecific dead cell staining we employ the viability dye, propidium iodide (Dangl and Herzenberg). Forward and right angle scatter are also recorded for each cell. The reagents employed in this study are sumarized in Table 1.

Table 1. Monoclonal antibody reagents used in these studies.

Clone	Determinant	Isotype	Conjugate	Reference
331.12	IgM	rat IgG2b	Fl	Kincade
53-7.8	Ly-1	rat IgG2a	bi	Ledbetter
53-6.7	Lyt-2	rat IgG2a	bi, apc	Ledbetter
M1/70	MAC-1	rat IgG2b	Fl	Springer
RA3-6B2	Ly-5(B220)	rat	Fl, bi	Coffman
49-H4	ThB	rat IgG2c	Fl, bi	Eckhardt
10-3.6	IA(Ial7)	mouse IgG2a	Fl	Oi
MK-D6	IA(d)	mouse IgG2a	Fl	Kappler
10-4.22	IgD	mouse IgG2a	Fl, bi	Oi
53-10.1	BLA-1	rat IgG2c	Fl	Hardy, 1984
30-E2	BLA-2	rat IgG2b	bi	Hardy, 1984
187.1	Kappa	rat IgG2c	Fl	Yelton

As expected from previous two-color studies, about 2-4 percent of spleen cells in 4 week old mice are Ly-1$^+$, Ly-2$^-$, IgM$^+$. Furthermore, these cells express high levels of ThB, but rather heterogenous levels of Ly-5(B220). Most Ly-1 B cells in spleen are thus IgM$^+$, ThB$^+$, Ly-5(B220)$^+$. Similar analyses of bone marrow suspensions demonstrate that while IgM$^+$ cells expressing Ly-1 are very rare, ThB$^+$ cells expressing Ly-1 are somewhat less rare, and that about 10 percent of the Ly-5(B220)$^+$ cells also express Ly-1. Staining for the macrophage-associated antigen defined by the MAC-1 monoclonal antibody is essentially at background in the Ly-1 B population in either spleen or bone marrow. These results are sumarized in Table 2.

Table 2. Antigen expression on Ly-1$^+$/Ly-2$^-$ cells in 4 week old NFS mice.

Tissue	Antigen	% of total cells (50K) exp 1	exp 2	Level of expression
Bone Marrow	Ly-5(B220)	0.48	2.50	low
	ThB	0.40	1.60	high
	IgM	0.18	1.00	heterogenous
	MAC-1	0.10	0.08	
Spleen	Ly-5(B220)	3.8		low/high (50/50)
	ThB	3.6		high
	IgM	3.0		high
	MAC-1	0.13		

This data, while not conclusive, suggests that the $Ly\text{-}1^+,IgM^+$ cells we have studied previously in spleen express the Ly-1 antigen as early as the $Ly\text{-}5(B220)^+$ pre-B stage. Indeed, although the numbers are quite small, the trend of more $ThB^+/Ly\text{-}1^+$ cells than $IgM^+/Ly\text{-}1^+$ cells and more $Ly\text{-}5(B220)^+/Ly\text{-}1^+$ cells than $ThB^+/Ly\text{-}1^+$ cells follows a B cell differentiation scheme described by Coffman for the bulk of the B cells in bone marrow. The significant point that we make here is that a functionally distinct population of splenic B cells (Ly-1 B) is phenotypically distinct from the bulk of bone marrow cells as early as the pre-B cell stage.

LY-1 EXPRESSION IN B CELL NEOPLASMS

The likely presence of Ly-1 on some pre-B cells and our previous demonstration of Ly-1 on certain antibody secreting cells suggests that the Ly-1 antigen marks a subpopulation of B cells at several different stages of differentiation. In order to investigate this more thoroughly, we have examined the phenotype of a number of B cell lines that can be grown in cell culture. We present the results of a series of Ly-1 bearing cell lines in table 3. As can be readily seen, this list includes cell lines previously typed as representing several distinct B cell differentiation stages.

Table 3. Ly-1 bearing B cell tumors and long term cultured cell lines.

Tumor or Cell Line		Characteristics	Light Chain
70Z/3	Pre-B;	IgM^-, $Ly\text{-}1^+$ --LPS--> IgM^+, $Ly\text{-}1^+$	Kappa
NFS-5	Pre-B;	IgM^-, $Ly\text{-}1^{+/-}$ --in vitro--> IgM^+, $Ly\text{-}1^+$	Kappa
WEHI-231	Early-B;	IgM^+, $Ly\text{-}1^+$, IA^-, IgD^-	Kappa
CH-1	B cell;	IgM^+, $Ly\text{-}1^+$, IA^+, IgD^-	Lambda
BCL_1	B cell;	IgM^+, $Ly\text{-}1^+$, IA^+, IgD^+	Lambda
NFS-1.0	Ly-1 B;	IgM^+, $Ly\text{-}1^+$, IA^+, IgD^+ inducible to make BrmRBC plaques	Lambda
NZB NB	Ly-1 B;	IgM^+, $Ly\text{-}1^+$, IA^-, IgD^- inducible to make BrmRBC plaques	Lambda
BALB/c NB	Ly-1 B;	Similar to NZB NB	Lambda

Certain of these cell lines have rather low levels of Ly-1 (WEHI-231) and we have noted some variability in Ly-1 expression with different isolates of another (70Z/3). However all of these lines stain above background (defined by isotype controls) for Ly-1.

LPS-INDUCED DIFFERENTIATION TO BROMELAIN PFC IN A LY-1 B TUMOR

Of all the lines examined, one (NFS-1.0) showed very low, but detectable reactivity with bromelain-treated mouse red blood cells. This line was cultured for 4 days in medium containing 10 micrograms/ml LPS and

then examined for surface phenotype and for bromelain PFC. Although the surface levels of IgM and Ly-1 were essentially unchanged from control cultures, there was a very large increase in the frequency of bromelain PFC (see table 4). This result demonstrates that the NFS-1.0 cell line is very similar to the Ly-1 B population in normal and NZB mice, not only in surface phenotype, but also in responsiveness (to LPS) and in specificity (for bromelain mouse RBC).

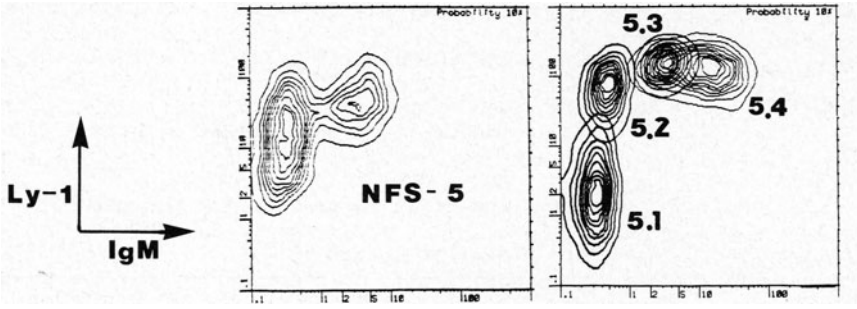
Table 4. The NFS-1.0 cell line is inducible with LPS to make bromelain PFC.

Cell Line	PFC/10^5 when cultured	
	without LPS	with LPS
NFS-1.0	10	3000
70Z/3	<1	<1

IN VITRO DIFFERENTIATION OF A LY-1 PRE-B CELL LINE

Examination of one of the "pre-B" Ly-1 bearing cell lines (NFS-5) showed considerable heterogeneity in the level of Ly-1 expression and, in addition, a small subpopulation of IgM^+ cells. Two color staining for IgM and Ly-1 demonstrated that the IgM^- population was very heterogenous for Ly-1 level (over at least a 100-fold range) whereas the IgM^+ population was uniformly high for Ly-1 expression (see figure 1). Subsequent FACS cloning of the three phenotypic Ly-1/IgM populations of NFS-5 has yielded large numbers of cells that stably are $Ly\text{-}1^-/IgM^-$, $Ly\text{-}1^+/IgM^-$ and $Ly\text{-}1^+/IgM^+$. Unexpectedly, we find that the $Ly\text{-}1^-/IgM^-$ cell line spontaneously becomes $Ly\text{-}1^+/IgM^+$ after culture for several weeks in medium containing horse serum, but not in medium containing FCS. The transition is even faster for the $Ly\text{-}1^+/IgM^-$ cell line.

Figure 1. Two color analysis of various NFS-5 isolates.



Recently the $Ly\text{-}1^+/IgM^+$ NFS-5 line has been shown to express yet another surface marker combination after culture in LPS. These cells were cultured in LPS for 4 days and then stained with a monoclonal antibody specific for Kappa light chain. The $Kappa^+$ cells were cloned into LPS-containing medium and several clones were isolated. Analysis of these clones demonstrated that although all clones were Kappa positive (with higher surface levels of IgM than the parent culture), they exhibited variable levels of a B cell differentiation antigen know as ThB (Eckhardt and Herzenberg). All prior cultures of NFS-5 were uniformly negative for ThB. The various stages of NFS-5 are sumarized in table 5.

Table 5. NFS-5 differentiation stage phenotypes.

Cell Line	Ly-1	IgM	Kappa	BLA-1	BLA-2	ThB	IA	IgD
NFS-5.1	+/-	-	-	+	-	-	-	-
NFS-5.2	++	-	-	+	-	-	-	-
NFS-5.3	+++	+	+/-	+	+	-	-	-
NFS-5.4	+++	++	+	+	+	+	-	-

LY-1 EXPRESSION ON "NORMAL" B CELL LINES

There have been numerous attempts to culture "normal" B cells from the bone marrow or spleen of various mice strains, but most have been unsuccessful. Recently, there have been several reports of successful cultivation of either bone marrow (Whitlock and Witte) or splenic (Braun) B cells. Davidson, following the protocol of Braun has established several splenic B cell lines and has observed that all are $Ly\text{-}1^+$ (see report in this volume). We have investigated two of these lines, one derived from BALB/c and one from NZB, for the expression of a number of B cell surface antigens. The results are summarized in table 3. Both of these lines have identical surface phenotypes for all markers examined to date, and, with the exception of IA and IgD, are very similar to the NFS-1.0 tumor line. Curiously, both cell lines express lambda light chains, a rare class (about 5 percent of IgM^+ cells in spleen) in mouse.

CONCLUSIONS

It appears that the expression of Ly-1 on a subpopulation of B cells occurs as early as the $Ly\text{-}5(B220)^+$, IgM^- pre-B stage in bone marow. This finding is corroborated by the demonstration of Ly-1 on pre-B type tumors (Davidson et al, 1984). One unusual specificity of splenic Ly-1 B (for bromelain-treated mouse erythrocytes) is also found with one of the Ly-1 bearing tumors (NFS-1.0). Finally, one Ly-1 bearing pre-B tumor shows regulated expression of a number of B cell differentiation antigens *in vitro*. The sequence of surface antigen expression in this line suggests that Ly-1 is a very early marker on cells of the Ly-1 B lineage.

The presence of Ly-1 on several "spontaneous" B cell lines established by long term culture of whole spleen is not wholely unexpected, considering early findings that Ly-1 B cells in spleen survived *in vitro* much better than $Ly\text{-}1^-$ B cells (Hayakawa et al, 1983). On the other hand, the finding that many of these Ly-1 B tumors and lines express the lambda light chain is surprising. Although there is some enrichment for lambda light chain expression in splenic Ly-1 B (compared with $Ly\text{-}1^-$ B cells), most Ly-1 B cells have kappa light chains (unpublished observations). Perhaps the expression of lambda light chain confers on the Ly-1 B cell a susceptibility to relatively unrestrained growth.

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